Five-Minute Oral Statement

[SLIDE 1] Good afternoon. I am Linda Stetzenbach the Director of the Microbiology Division at the Harry Reid Center for Environmental Studies at the University of Nevada, Las Vegas where I have been conducting research related to airborne and surface-associated microorganisms for more than 17 years.

[SLIDE 2] In 1994 I presented results of our laboratory's work on the dispersal of bacteria from flooring materials into the air at a scientific conference on chemical and biological defense research at Aberdeen Proving Ground, Maryland. The bacteria that we used were simulants for the causative agent of the disease anthrax. During a break after my presentation I was told by an attendee that while my data were interesting, anthrax spores would not become airborne once they settled so my research was of little interest to biodefense. Unfortunately, the events of 2001 demonstrated the dispersal of infectious spores from letters and postal machinery, and that re-aerosolization of settled microorganisms does occur.

[SLIDE 3] Since 2001, it is also acknowledged that monitoring for biothreat agents is problematic due to the lack of standardized sampling and analysis protocols. For example, a variety of surface sampling methods has been used by various governmental agencies when monitoring for biothreat agents, but the likelihood of success using these sampling methods has not been established, and there are currently no standardized environmental sampling methods for first responders, public health officials, law enforcement and other agencies.

[SLIDE 4] Surface sampling is important for determining the presence and concentration of a contaminant, the location where an agent may have been released, the extent of contamination, forensics, and the effectiveness of remediation procedures.

[SLIDE 5] While swab sampling has a time honored tradition in the hospital setting for everything from sore throats to wounds, the usefulness of this method for sampling buildings is limited. One disadvantage is the large number of samples that can be generated due to the small surface areas that are sampled. For example, tens of thousands of swab samples were collected as a result of the anthrax attacks and the laboratory response network was overwhelmed with samples.

[SLIDE 6] Handling of swabs by emergency personnel responding to a suspected incident is also difficult.

[SLIDE 7] Results in our laboratory of a government-developed large area surface sampler (the BiSKit) demonstrated an ability to rapidly sample a large area, which translates into better detection and fewer samples, but the swab and the BiSKit are the only two surface sampling methods that have undergone validation testing. However, validation and the establishment of protocols used to determine if a biothreat exists in a building are critical.

[SLIDE 8] Therefore, research should be conducted to evaluate currently available and newly developing devices for biological sampling of surfaces. This would provide information on their

efficiency of collection and the limits of their capability; information that can be used to determine what device is optimal for which biothreat scenario.

[SLIDE 9] An integral part of this research is developing analytical capability through the application of molecular biology methods that enhance the enumeration and characterization of biothreat agents.

[SLIDE 10] Molecular biology methods are rapid, sensitive, and specific,

[SLIDE 11] but there are potential interferences resulting from the presence of environmental background material. Therefore, research is needed to minimize interferences, to optimize analysis of airborne and surface samples, and to develop standard operating procedures for optimal detection and measurement of biological contaminants on surfaces. Comprehensive research on these topics would enhance sampling capabilities for the purpose of identification or attribution while allowing inter-laboratory/inter-agency comparison of data.

[SLIDE 12] There are also serious concerns with assessing the results of bioaerosol monitoring in indoor and outdoor environments. The purposeful dissemination of biothreat agents in enclosed public environments and at outdoor facilities that attract the public would potentially result in the exposure of large numbers of individuals. Therefore, programs utilizing routine monitoring of bioaerosols have been initiated. Unfortunately, little information is available on the natural background populations of organisms designated as biothreat agents. This lack of data has resulted in false positive results with the Biowatch System that is currently deployed in selected cities in the United States. A comprehensive survey to determine the levels of naturally occurring biothreat agents would assist decision makers when interpreting positive detection results.

In addition, naturally-occurring microorganisms in the air and on surfaces can affect the ability to discriminate background aerosols from biocontaminants. While some data have been published, the naturally-occurring microorganisms in the types of facilities that may be sites of a purposeful biocontaminant release (such as sports arenas, convention facilities, and mass transit) have not been adequately characterized. Therefore, research should be conducted in a variety of public environments and outdoor facilities to characterize background populations of airborne and surface-associated microorganisms that can be dispersed and interfere with the measurement of purposefully released biocontaminants.

[SLIDE 13] The research that I have outlined for you today is vital to provide rapid and accurate information to decision makers that are charged with protecting the public health and security of our citizens. In closing, I wish to emphasize that universities are an underutilized resource for much of this research. University scientists have a track record for high quality research on these topics and are they are cost effective. In addition, they do not have a vested interest in any particular method and can develop and evaluate protocols with an unbiased perspective.

[SLIDE 14] I would be happy to answer any questions that members of this committee may have. Thank you.

Full Statement

Linda D. Stetzenbach, Ph.D.
University of Nevada, Las Vegas

Biothreat agents, such as the spores of *Bacillus anthracis* that cause anthrax, can be efficiently dispersed in aerosols. The spores eventually settle onto surfaces as occurred at the Hart Senate Office building in Washington, D.C. in October 2001. Research has shown that surface-associated biocontaminants can become re-aerosolized, resulting in exposure to building occupants, but detection of airborne and surface-associated biological agents is problematic. Air sampling is useful in determining the concentration and populations of microorganisms in the air at the time samples are collected. Similarly, surface sampling can be used to determine the presence and concentration of biocontaminants on solid and porous surfaces. Surface sampling can provide information on *i*) the location(s) where an agent may have been released, *ii*) the presence and concentration of a contaminant, *iii*) the extent of contamination, *iv*) forensics for law enforcement, and *v*) evaluation of the effectiveness of remediation procedures. However, while a variety of surface sampling methods has been used by various governmental agencies for the detection of biocontaminants, the sampling efficiency and lower detection limits for these sampling methods have not been established, and there are currently no standardized environmental sampling methods. There are no established, validated protocols for first responders, public health officials, law enforcement and other agencies to use when called upon to monitor a building suspected of experiencing a biothreat event. Only two surface sampling methods, the swab and the Biological Sampling Kit (BiSKit), have undergone validation testing. In a study published in 2004 four swab materials were evaluated for the efficiency of recovery of *B. anthracis* from the surface of steel coupons. The authors determined that the greatest recovery of spores was obtained with pre-moistened macrofoam and cotton swabs, and processing by vortexing to remove spores from the swabs. However, the disadvantages of swab sampling include the lack of sensitivity of detection and the large number of samples that can be generated due to the small surface areas sampled. For example, tens of thousands of swab samples were collected following the anthrax attacks and the laboratory response network was overwhelmed with samples. In our laboratory, the surface sampling efficiency of a government-developed large area surface sampling method, the BiSKit, was measured using *B. atrophaeus* (BG), a simulant for *B. anthracis*, and the data were compared with cotton and foam swab-based sampling. Results of this study published in 2004 showed that the primary advantage of the BiSKit was its ability to rapidly sample approximately 1 square yard areas compared to small areas (16 in^2 or 49 in^2), and that the number of bacteria sampled with the BiSKit was 10 times higher. This translates into greater sensitivity of detection and generates fewer samples. Unfortunately, these two studies are the only published data on surface sampling efficiency methods, yet validation and establishment of protocols used to determine if a biothreat exists in a building are vital. Therefore, research should be conducted 1) to evaluate currently available devices for biological sampling of surfaces, 2) to determine their collection efficiencies and sensitivities, 3) to test sample processing methods to enhance retrieval of biothreat agents and to remove inhibitory compounds while minimizing losses of target DNA, and 4) to establish standard operating procedures for optimal detection and measurement of biological contaminants on surfaces. This research would enhance sampling capabilities for the purpose of identification or attribution while allowing inter-laboratory/inter-agency comparison of data.

An integral part of this research is developing analytical capability through the application of molecular biology methods that enhance the quantification and characterization of biothreat agents. These methods are rapid, sensitive, and specific, but there are potential interferences resulting from the presence of environmental background material. Researchers have demonstrated that simple house dust can interfere with molecular detection of biocontaminants and that this interference can be overcome, but protocols for sample cleanup have not been established.

Research described above should be performed in a phased approach including both laboratory and bioaerosol release experiments conducted in room-sized experimental chambers. In the first phase, laboratory experiments should be conducted to determine the overall collection efficiency and sensitivity of a variety of currently available surface sampling methods. Representative methods for sampling large areas, small areas and textured surfaces should be tested. Surfaces should include smooth materials (e.g., plastic and painted metal), semi-porous materials (e.g., wood laminate and vinyl tile), and textured surfaces (e.g., fabric and carpet). Sample processing alternatives should be quantitatively evaluated and incorporated into the test protocol to maximize detection of the target organisms. Solutions to interference with molecular detection methods resulting from environmental background (e.g., settled dust) should be investigated by testing sample cleanup methods. The resulting protocols developed from the laboratory experiments should then be validated in research chamber experiments in which bioaerosols are generated, allowed to deposit on test materials, and the surface sampling and analysis is conducted. Innovative and/or newly developed surface sampling methods should be evaluated in laboratory and the research chamber as they are developed. Written Standard Operating Procedures should be developed for the sampling and analysis protocols and a training program for practitioners should be developed and conducted.

There are also serious concerns with assessing the results of bioaerosol monitoring in indoor and outdoor environments. The purposeful dissemination of biocontaminants in enclosed public environments and at outdoor facilities that attract the public would potentially result in the exposure of large numbers of individuals to biothreat agents. However, little information is currently available on the natural background populations of organisms designated as biothreat agents. This lack of data has resulted in false positive results with the Biowatch System that is currently deployed in selected cities in the United States. A comprehensive survey to determine the levels of naturally occurring biothreat microorganisms would assist decision makers when interpreting positive results. In addition, the highly variable composition and concentration of indigenous microorganisms in the air and on surfaces can affect the ability to discriminate background aerosols from biocontaminants. While some data have been published on bioaerosols in agricultural settings, compost facilities, office buildings, schools, and residences, the naturally occurring microorganisms in the types of facilities that may be sites of a purposeful biocontaminant release (e.g., sports arenas, convention facilities, and mass transit system terminals) have not been adequately characterized and interference due to the re-distribution of settled microbial contaminants into the air in these facilities as a result of human activity has not been measured. Therefore, research should be conducted to monitor background populations of airborne microorganisms in a variety of public environments and outdoor facilities, including surface-associated organisms that can be dispersed and interfere with the measurement of purposefully released biocontaminants.

More than 25 scientific papers on bioaerosols, surface sampling, and enhanced detection of microorganisms have been published papers since 1991 by scientists at the University of Nevada, Las Vegas. The following are citations of some of those papers.

Alvarez, A.J., M.P. Buttner, and L.D. Stetzenbach. 1995. PCR for bioaerosol monitoring: sensitivity and environmental interference. Applied and Environmental Microbiology, Vol. 61, pp. 3639-3644.

Buttner, M.P., P. Cruz-Perez, L.D. Stetzenbach, A.K. Klima-Comba, V.L. Stevens, and P.A. Emanuel. 2004. Evaluation of the Biological Sampling Kit (BiSKit) for large-area surface sampling. Applied and Environmental Microbiology, Vol. 70, pp. 7040-7045.

Buttner, M.P., P. Cruz, L.D. Stetzenbach, A.K. Klima-Comba, V.L. Stevens, and T.D. Cronin. 2004. Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis. Applied and Environmental Microbiology, Vol. 70, pp. 4740-4747.

Buttner, M.P., P. Cruz-Perez, and L.D. Stetzenbach. 2001. Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. Applied and Environmental Microbiology, Vol. 67(6), pp. 2564-2570.

Buttner, M.P., and L.D. Stetzenbach. 1993. Monitoring of fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. Applied and Environmental Microbiology, Vol. 59, pp. 219-226.

Stetzenbach, L.D., M.P. Buttner, and P. Cruz. 2004. Detection and Enumeration of Airborne Biocontaminants. Current Opinion in Biotechnology, Vol. 15, pp. 170-174.

Stetzenbach, L.D., A.J. Alvarez, and M.P. Buttner. 1996. The Use of Polymerase Chain Reaction (PCR) to Enhance Bioaerosol Monitoring. *In* D.A. Berg (ed.), Proceedings of the 1994 ERDEC Scientific Conference on Chemical and Biological Defense Research. Aberdeen Proving Ground, MD.